



Protein Quantification Reagents and Protocols

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[Required Reagents and Equipment]

- 96-well microplate (flat bottom)
- Microplate reader
- [Standard Solution of Albumin from Bovine Serum](#) (TCI Product No. **T3796**)
- [Bradford Assay Solution \(Ready-to-use\) \[for Protein determination\]](#) (TCI Product No. **B5702**)
or [Pyrogallol Red \(Ready-to-use solution\)](#) (TCI Product No. **P2575**)

[Preparation Before the Experiment]

1. Allow all reagents to equilibrate to room temperature.
2. Gently mix each reagent until homogenous.

[Preparation of BSA Standard Solutions]

Prepare seven sample tubes and make a BSA dilution series as shown in the table below.

Tube No.	Conc.	How to prepare
1	1000 µg/mL	Standard Solution of Albumin from Bovine Serum (TCI Product No. T3796) 50 µL + purified water (or PBS) 50 µL
2	500 µg/mL	50 µL from tube 1 + purified water (or PBS) 50 µL
3	250 µg/mL	50 µL from tube 2 + purified water (or PBS) 50 µL
4	125 µg/mL	50 µL from tube 3 + purified water (or PBS) 50 µL
5	62.5 µg/mL	50 µL from tube 4 + purified water (or PBS) 50 µL
6	31.25 µg/mL	50 µL from tube 5 + purified water (or PBS) 50 µL
7	0 µg/mL	purified water (or PBS) 100 µL

[Protein Quantification]

(A) Bradford Method

The Bradford method is a protein quantification technique that takes advantage of Coomassie Brilliant Blue G-250's absorbance peak shift from 465 nm to 595 nm upon protein binding. It allows for detection of proteins down to 1.0 µg/mL in a very short amount of time.

1. Add 4 µL of BSA standard solution or sample to each well of a microplate.
2. Add 200 µL Bradford Assay Solution (Ready-to-use) [for Protein determination] (TCI Product No. **B5702**) to each well and incubate at room temperature for 5 minutes.
3. Measure the absorbance at 595 nm using a microplate reader.

(B) Pyrogallol Red Method

The Pyrogallol Red (PR) method is a protein quantification technique that takes advantage of the absorbance peak shift of a PR-molybdenum complex from 480 nm to 600 nm upon protein binding. It enables the measurement of various protein concentrations with minimal interference from other solutes.

1. Add 10 μL of BSA standard solution or sample to each well of a microplate.
2. Add 200 μL of Pyrogallol Red (Ready-to-use solution) (TCI Product No. **P2575**) to each well and incubate at room temperature for 30 minutes.
3. Measure the absorbance at 600 nm using a microplate reader.

[Data Analysis]

1. Create a calibration curve with the corrected values (obtained by subtracting the blank absorbance [tube 7] from the absorbance of each dilution series [tubes 1-6]) on the vertical axis and the BSA concentration on the horizontal axis.
2. Calculate the protein concentration ($\mu\text{g/mL}$) from the slope and intercept of the graph and the absorbance of the corrected sample.